Laboratory Manager Approval: Mary K. Bowman / 08/19/2021

QA Manager Approval: Jeffrey Moore / 08/19/2021

Nitrogen and Phosphorus containing Pesticides-EPA Method 507

Access to this SOP shall be available within the laboratory for reference purposes; the official copy of this SOP resides on the official Georgia EPD website at https://epd.georgia.gov/about-us/epd-laboratory-operations. Printed copies of this SOP will contain a watermark indicating the copy is an uncontrolled copy.

1. Scope and Application

1.1. Method 507 is used to determine the concentrations of nitrogen and phosphorous pesticides in drinking water. Samples are extracted at neutral pH with methylene chloride. The extract is analyzed by injection into a temperature programmable gas chromatograph with a nitrogen-phosphorus detector. Identifications are obtained by analyzing a standard curve under identical conditions used for samples and comparing resultant retention times. Concentrations of the identified components are measured by relating the response produced for that compound to the standard curve response.

1.2. This method is restricted to analysts who have completed the requirements of the initial demonstration SOP. Refer to 13.2.

2. Definitions

2.1. Refer to Section 3 and Section 4 of the Georgia EPD Laboratory Quality Assurance Manual for Quality Control definitions.

2.2. Primary Source (PS) – A standard that is used to make up the calibration points of a curve.

2.3. Second Source (SS) – A standard made from another manufacturer other than that of the primary source.

2.4. Initial Calibration Verification (ICV) – An ICV is a second source standard that is used to verify the correctness of the primary source's calibration curve. The ICV is run at a level equal to that of a Laboratory Control Sample (LCS) or the mid-point on the calibration curve.

3. Interferences

3.1. Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baselines in chromatograms.

3.1.1. Glassware must be scrupulously cleaned with hot water and detergent followed by de-ionized water then rinsed with methanol followed by acetone. The glassware is rinsed again with extraction solvent, methylene chloride, immediately prior to use.

3.1.2. The use of high purity reagents and solvents helps to minimize interference problems.

3.2. Interfering contamination may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes.

3.3. Matrix interferences may be caused by contaminants that are co-extracted from the sample.

4. Safety

4.1. Refer to Georgia EPD Laboratory Chemical Hygiene Plan.

5. Apparatus and Equipment

5.1. Sample container: 1-liter amber bottle with Teflon-lined caps

5.2. Vials: auto-sampler vials, screw top, 2 ml and 300 µl inserts

5.3. Volumetric flask: various sizes

5.4. Micro-syringes: various sizes

5.5. Syringes: various sizes

5.6. Drying column: sodium sulfate

5.7. Gas chromatograph: capable of temperature programming equipped for split/splitless injection

5.7.1. Mega bore 30 m X 0.53 mm, CLP-1 or equivalent (0.32 mm may be used)

5.7.2. Mega bore 30 m X 0.53 mm, CLP-2 or equivalent (0.32 mm may be used)

5.7.3. Nitrogen-phosphorus detector

5.7.4. Chromatography software

5.8. Separatory Funnel: 2 L with PTFE stopcock

5.9. Separatory Funnel Shaker

5.10. Graduated cylinders (Class A): various sizes

5.11. Erlenmeyer flasks: 250 ml – 300 ml

5.12. Beakers: various sizes

5.13. pH indicator paper: pH range including the desired extraction pH range

5.14. Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g

5.15. Balance: Top loading, capable of accurately weighing to the nearest 0.01 g; may be used for weighing Sodium chloride (see 6.6.)

5.16. RapidVap or similar concentrator with nitrogen blow down and controlled heating capabilities

5.16.1. RapidVap or similar concentration tubes with at least 300 ml volume

5.17. TurboVap or similar concentrator with nitrogen blow down and controlled heating capabilities

5.17.1. TurboVap or similar concentration tubes with at least 50 ml volume

5.18. Sample extract vials: culture tubes with at least 5 ml volume

5.19. Disposable pipettes and bulbs

5.20. Detergent: Steris Labklenz or equivalent

6. Reagents and Standards

6.1. Methylene chloride: pesticide grade or equivalent

6.2. Methyl tert-butyl ether: pesticide grade or equivalent

6.3. Acetone: pesticide grade or equivalent

6.4. Isooctane: pesticide grade or equivalent

6.5. Reagent water: Purified water which does not contain any measurable quantities of target analytes or interfering compounds for each compound of interest (deionized, HPLC, Milli-Q or equivalent). Milli-Q water has a resistivity of 18 MΩ·cm or greater @ 25° C and a TOC of 50µg/L or less.

6.6. Sodium chloride (salt), ACS reagent grade or equivalent

6.6.1. Sodium chloride is baked for 4 hours at 400° C then stored in a glass container

6.7. Sodium thiosulfate: granular, anhydrous, reagent grade or equivalent

6.8. Sodium sulfate: granular, anhydrous, certified ACS grade suitable for pesticide residue analysis or equivalent

6.8.1. Sodium sulfate is baked for 4 hours at 450° C then stored in a glass container

6.9. 1N HCl

6.9.1. An empty solvent bottle is rinsed with DI water then filled with 3600 ml of DI water. 400 ml of concentrated HCl (approximately 38% w/w) is carefully added and the resultant solution mixed thoroughly.

6.10. 1 M Dipotassium phosphate Solution

6.10.1. Weigh and transfer 174.18 g of neat (≥ 96% purity) di-potassium phosphate into a 1 L volumetric flask. Dilute to volume with DI water and mix thoroughly.

6.11. Phosphate Buffer

6.11.1. Combine 300 ml of 1 N HCl and 500 ml of 1 M Dipotassium phosphate in a 1000 ml volumetric flask (used for mixing only: do not fill to volume) for a final volume of 800 ml. Mix thoroughly.

6.12. Calibration Standard Solutions

6.12.1. Prepare four different concentrations equivalent to the concentration levels in Section 8.2. , by dilution of the stock standard solutions. Standard stock solutions are usually at a concentration of 100 µg/ml or 1000 µg/ml. Standards derived from neat compounds are also acceptable. Calculations or amounts will vary depending on the stock standard concentration. Prepare the primary dilution standard at 10 µg/ml concentration.

6.13. Initial Calibration Verification Standard Solutions (ICV)

6.13.1. Stock standard solutions prepared from a second source vendor's standards or a different lot from the same vendor as the calibration standards containing all of the analytes listed in Section 8.2. , diluted in MtBE. This standard is equivalent to Level 2 calibration standard in concentration.

6.14. Spiking Solution

6.14.1. 10 µg/ml in acetone, spiking volume 100 µl. 508 single component compounds included at 1 µg/ml.

Table 6.14. 1 – 507 Spiking Stock Standards in Acetone

Compound	**Initial Concentration (µg/l)**	**Aliquot (ml)**	**Final Concentration (µg/ml)**
Alachlor	100	1.0	10
Atrazine	100	1.0	10
Simazine	100	1.0	10
Total Volume of Standard Aliquots	1.0 ml		
Addition of Acetone to Standard Aliquots	9.0 ml		
Final Volume of Spiking Stock Standard in Acetone	10 ml		

Table 6.14. 2 – 507 Spiking Standards in MtBE

Compound	**Initial Concentration (µg/ml)**	**Aliquot**	**Final Concentration (µg/ml)**
Alachlor	10	100 µl (Use one 100 µl aliquot of the Spiking Stock in each sample. Spiking stock contains all of the compounds listed to the left.)	0.20
Atrazine	10		0.20
Simazine	10		0.20
Final volume of Spiking Stock Standard is 5mls in MtBE (sample extract)			

6.15. Surrogate Spiking Solution

6.15.1. NMX (1,3-Dimethyl-2-nitrobenzene) 100 µg/ml and 4 µg/ml TCMX (Tetrachlor-m-xylene for 508 analysis) in acetone, spiking volume 100 µl.

6.15.2. NMX at 1000 µg/ml is made from neat standard, 96% purity, or better. 0.100 g is weighed in a 100 ml volumetric flask using a certified analytical balance and then brought to final volume with acetone.

6.15.3. TCMX is typically a certified stock standard at 2000 µg/ml.

6.15.4. 5 ml of the 1000 µg/ml NMX and 100 µl of the 2000 µg/ml TCMX are added to a 50 ml volumetric flask and brought to final volume with acetone.

6.15.5. Should an alternate concentration of surrogate standards be required, or neat standard used due to availability issues, the calculations and/or final volumes will be modified to meet the surrogate concentrations NMX 100 µg/mL and TCMX 4 µg/ml.

6.16. All standards that are made for the 507 analysis are to have an expiration date of two months from the opening of the vendor stock ampule or the manufacturer's expiration date if less than two months from opening.

7. Sample Collection

7.1. Drinking water samples for EPA Method 507 are collected in four amber, pre-certified 1000 ml glass bottles with Teflon lined screw caps and preserved with 80 mg of Sodium thiosulfate. The 80 mg of Sodium thiosulfate is sufficient to neutralize up to 5 mg/L (ppm) residual chlorine.

7.2. A residual chlorine check is done in the field by the collector. The collector writes down the numerical value for residual chlorine in ppm on the sampling form.

7.3. The shipping and receiving staff log in the samples and enter the information for residual chlorine in the DNR_LAB Labworks field. The analyst prints a backlog to determine samples to be analyzed.

7.4. The backlog report contains the residual chlorine concentration determined by the collector. If the residual chlorine measured by the collector is less than 5 ppm, the 80 mg of Sodium thiosulfate in the bottle was sufficient to neutralize all of the residual chlorine in the sample.

7.4.1. If the collector reports 5 ppm or more residual chlorine, the sample must be recollected.

7.5. Samples are cooled to 0 - 6°C (not frozen) after sample collection. Four bottles are to be collected for every sample. Samples must be extracted within 7 days from collection and analyzed within 14 days of extraction.

8. Calibration

8.1. Calibration Curve

8.1.1. A four-point calibration is performed for all components. The calibration system uses traceable certified standards. The calibration is an external standard calibration with an average of response factor linear curve fit or quadratic fit and should result in a percent relative standard deviation < 20% between calibration levels of each analyte. Alternatively, the calibration curve may be a least squares regression.

8.2. Calibration Standards

8.2.1. The calibration curve consists of the calibration standards at the following concentrations (µg/ml) diluted from 10.0µg/ml stock (see table 6.14.1). NMX is diluted from 1000µg/ml stock (see 6.15.2.):

Table 8.2. 1 - 507 Calibration Curve Levels (µg/ml) Note: All standards are diluted to a final volume of 10 mL in MtBE

Compound	Level 1	Level 2	Level 3	Level 4
Alachlor	0.030	0.200	0.300	1.00
Atrazine	0.030	0.200	0.300	1.00
Simazine	0.030	0.200	0.300	1.00
NMX - SS	2.00	2.00	2.00	2.00

Table 8.2. 2 - Aliquots of 507 Calibration Stock to make up all the levels in Table 8.2. 1

(Aliquots corresponds to each level directly above each column)

	Level 1	Level 2	Level 3	Level 4
Aliquot of 507 Calibration Stock (see Table 8.2. 1)	0.030 ml (or 30 µl)	0.200 ml (or 200 µl)	0.300 ml (or 300 µl)	1.00 ml (or 1000 µl)
NMX stock	200 µl	200 µl	200 µl	200 µl
Bring all levels (points of the curve) up to 10 ml by using MtBE				

8.3. Calibration Verification

8.3.1. Second source calibration verification (ICV) must be analyzed after initial calibration and at least once per quarter even if the system has not been recalibrated. All analytes must be within ± 20% of the expected value.

8.3.2. A daily continuing calibration of alternating levels is performed every eight-hour analysis period to monitor and validate the instrumentation, column, and detector performance.

8.3.3. An LPC (Laboratory Performance Check) standard is run at the beginning of each sample sequence prior to the analysis of samples to determine sensitivity, chromatographic and column performance. The LPC criteria are calculated and reported by Total Chrom or equivalent chromatography software or manually. See calculation 11.13.

8.4. Record Keeping

8.4.1. Documentation of an instrument calibration is reviewed for adherence to quality criteria and archived with project records.

8.5. Daily Calibration Verification and Continuing Calibration

8.5.1. A continuing calibration standard (CCC) ensures the instruments target compound retention times and quantitation parameters meet method performance criteria. A CCC must be analyzed prior to sample analysis, one every 8 hours, and one at the end of the analysis sequence. Continuing calibration standards are analyzed during the analysis period to verify that instrument calibration accuracy does not exceed 20% of the initial calibration, i.e. %Drift ≤ 20% (calculation 11.7.). If the continuing calibration does not meet method performance criteria, then the instrument must be recalibrated. Two levels of calibration standards are alternated throughout the run. A CCC is required after running the standard curve and initial calibration verification. Note: After running a calibration curve, the ICV may be substituted for the initial CCC if it meets method criteria.

8.6. Average Response Factor Calibration

8.6.1. To evaluate the linearity of the initial calibration, calculate the mean response factor (RF), the standard deviation (σ_{n-1}) and the relative standard deviation expressed as a percentage (%RSD). If the %RSD of the response factors is ≤ 20% over the calibration range, then linearity through the origin may be assumed, and the average calibration or response may be used to determine sample concentrations. See calculations 11.1. – 11.3.

8.7. Linear Calibration using First Order Least Squares Regression

8.7.1. Linearity through the origin is not assumed in a least squares fit. The instrument responses versus the concentration of the standards for the 4 points are evaluated using the instrument data analysis software. The regression will produce the slope and intercept terms for a linear equation. The regression calculation will regenerate a correlation, r, a measure of goodness of fit of the regression line to the data. A value of 1.0 is a perfect fit. An acceptable correlation of coefficient should be $r \geq 0.990$ (or $r^2 \geq 0.980$). See calculation 11.4.

8.7.2. Alternatively, second order quadratic fit may be used with an acceptable correlation of coefficient of $r \geq 0.990$ (or $r^2 \geq 0.980$). Note: quadratic fit will be calculated by chromatographic software. See calculation 11.5.

8.8. Retention Time Windows

8.8.1. The width of the retention time window for each analyte, surrogate and major constituent in multi-component analytes is defined as ± 3 times the standard deviation of the mean absolute retention time of CCCs established over the approximate time period needed for a 20 sample analytical batch sequence. The CCCs (all levels analyzed) for an actual 20 sample batch (or smaller batch with sufficient injections afterwards (solvent blanks and CCCs) to simulate a 20 sample batch) may be used. See calculations 11.6.

8.9. Daily Retention Time Update

8.9.1. Retention Times (RT) are updated once per analytical sequence when ran on a GC for 507 analysis. Due to NP bead instability, an initial calibration is run with each batch then processed using Totalchrom or equivalent chromatography software. The RTs are updated at the time of calibration using the ICV. If a calibration is used over a one day cycle, an existing Totalchrom method extension is added by using "Month-Day-Year" and the initial daily CCC to reset RTs for that day. Hard copies of the updated calibration parameters are added to the data package for that batch of samples along with the original calibration.

8.10. Verification of Linear Calibrations

8.10.1. Calibration verification for linear calibrations involves the calculations of %Drift of the instrument response between the initial calibration and each subsequent analysis of the verification standard. The %Drift may be no more than ± 20%. See calculation 11.7.

8.11. Sample Concentration

8.11.1. Sample results are expressed in µg/L.

8.11.2. If an analyte response is calibrated by Average Response Factor, $\overline{RF}$, the chromatographic software calculates the concentration of the extract per calculation 11.8. Calculations are in µg/ml.

8.11.3. If an analyte response is calibrated by linear regression, the chromatographic software calculates the concentration of the extract solving for "x" per calculation 11.4. Calculations are in µg/ml.

8.11.4. If an analyte response is calibrated by quadratic fit, the chromatographic software calculates the concentration of the extract solving for "x" per calculation 11.5. Calculations are in µg/ml.

8.11.5. The sample concentration is calculated per calculation 11.9. Calculations are in µg/L. Assuming a 1000 ml initial sample volume and a 5 ml extract volume, calculation 11.9. can be reduced to Cs multiplied by a factor of 5.0. The chromatographic report uses this factor to multiply the result from either paragraph 8.11.2. , 8.11.3. or 8.11.4 above and calculates the final result per calculation 11.9.

8.11.6. If an initial volume of other than 1000 ml is used or a dilution of the extract is analyzed, the final sample result is multiplied by the factor determined with per calculation 11.10.

9. Quality Control

9.1. Refer to Table 14.1. 1 for Reporting Limits (RLs), Appendix A for Quality Assurance criteria and Table 14.1. 2 for a summary of Quality Control procedures associated with this method.

9.2. A Method Detection Limit Study for all analytes must be performed once per year. Refer to 13.6.

9.3. Refer to 13.2. for training and certification procedures.

9.4. Refer to 13.3. for control charting procedures.

9.5. Default control limits for recovery are based on Section 9.3.2 and Table 2 of EPA Method 507 (Section 9.5.1 for surrogate recovery). See 13.1. Precision limit defaults are set by the EPD Laboratory. In-house limits based on control charts may never exceed the default limits. These control limits are presented to assist in defining control limits established with control charts and are not used as batch acceptance criteria.

9.6. LCS control limits are used to monitor LCSD recovery. LCSD recovery is not used to validate batch data; however, LCS/LCSD precision (%RPD) is used for batch validation.

Note: Analysts must use the control limits presented in Appendix A, Table A.1. Those limits cannot exceed the default limits presented in Table 9.6. 1.

Table 9.6. 1 - Default QC Limits

	Compound	Default LCL %Recovery	Default UCL %Recovery	Default Precision %RPD
LCS/LCSD				
	Alachlor	60	110	30
	Atrazine	64	120	30
	Simazine	70	130	30
Surrogate*				
	NMX (Surrogate)	70 (7 µg/L)	130 (13 µg/L)	NA
MS/MSD*				
	Alachlor	55	115	30
	Atrazine	60	124	30
	Simazine	65	135	30

Note: Analysts must use the control limits presented in Appendix A, Table A.1. Those limits cannot exceed the default limits presented in Table 9.6. 1.

Table 9.6. 1 - Default QC Limits

*Method 507 specifies a static surrogate recovery range of 70 – 130% and static matrix spike recovery ranges of R ± 35%. The EPD Lab sets a static matrix spike precision range of 0 – 30%.

Note: Analysts must use the data in Appendix A, Table A.1; Table 9.6. 2 is presented as information on how the default control limits in Table 9.6. 1 are established.

Table 9.6. 2 EPA 507 Defined Control Limits *

Compound	R (% Recovery)	-30% R (LCS/LCSD)	+30% R (LCS/LCSD)	-35%R (MS/MSD)	+35%R (MS/MSD)
Alachlor	85	60	110	55	115
Atrazine	92	64	120	60	124
Simazine	100	70	130	65	135

*Defined by EPA Method 507.

9.7. MS/MSD pairs are analyzed at a minimum of 5% of all samples analyzed over time (one MS/MSD pair per 20 samples).

9.8. Performance Test (PT) Sample:

9.8.1. EPA requires that the Laboratory perform a PT sample every 12 months to maintain certification in EPA method 507. Those PT result must fall within acceptable control limits for the PT testing facility. If those results are not within acceptable control limits the Laboratory will have a second chance to pass the PT study within the same 12 months of the study. If the results did not fall within acceptable control limits for the study over the 12-month testing period, the laboratory will be downgraded for those compounds listed in this SOP. With the failure of this nature the laboratory must notify all drinking water facilities within 30 days of the failure after the 12-month period has passed. It is not until the laboratory passes a PT study will the laboratory be able to test for those compounds of interest again.

9.9. Method Detection Limit Study (MDL):

9.9.1. MDL is the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.

9.9.2. The actual MDL varies depending on instrument and matrix.

9.9.3. The MDL must be determined annually for each instrument prior to results being reported for that instrument. The MDL determined for each compound must be less than the reporting limit for that compound.

9.9.4. An MDL study may be done two different ways. The two different ways are considered and initial MDL study and a continuous MDL study. Both ways will be explained below.

9.10. Initial MDL study:

9.10.1. An initial MDL study may occur when a new instrument is brought online, changes to the method (which affect the compound of interest's peak area), and lastly major instrument repairs have been made.

9.10.2. An initial MDL study will consist of the following operating parameters, 7 MDL samples and 7 MDL blanks. The 7 MDL samples study is performed by preparing 7 spiked vials, MDLSpike, spiked at the lowest calibration point of the curve, and preparing 7 clean blank vials filled with DI water, MDLBlank. These 7 sets of spiked and blank vial "pairs" are analyzed over 3 separate days, there may or may not be a non-analysis day between each of the 3 days. A total of 14 vials are prepared, 7 spiked and 7 blanks.

9.11. Continuous MDL study:

9.11.1. A Continuous MDL study is preferred over the initial except in a few cases. For a continuous MDL study to be used on an instrument it must have a minimum of 7 MDL samples and 7 MDL blanks extracted over the course of multiple batches over a year. It is required that at a minimum 2 MDL samples and 2 MDL blanks must be ran per quarter per instrument. If this requirement is not met, then the initial MDL study must be performed for that instrument. (See section 9.10.2 for requirements.)

9.11.2. A continuous format MDL study is performed where one vial is spiked as an MDLSpike, at the lowest point of the calibration curve and analyzed with every batch of samples along with the method blank vial as an MDLBlank.

9.11.3. The results of the MDLBlank will be entered into Labworks using the Method Blank test code, $B_507A. The MDLSpike result will be entered using the $ML507A. The MDL Spiked Amount will be entered into the test code $MA507A. The instrument used for the MDL and Blank analysis will be selected using the test code INSTR-507A.

9.11.4. MDL studies must be pulled on a yearly basis or an initial MDL study must be performed before the current MDLs for the instrument expire.

10. Procedure

10.1. Create a batch consisting of a Blank, LCS/LCSD and MS/MSD and up to 20 samples.

10.2. Fill each of the sample bottles pertaining to the Laboratory Blank, Laboratory Control Sample (LCS), and Laboratory Control Sample Duplicate (LCSD) with de-ionized water.

10.2.1. The Blank is defined as de-ionized water spiked with 100 µl of surrogate solution (see Section 6.13.) + 80 mg of sodium thiosulfate in a 1 Liter amber bottle same as sample collection bottles. Mark the meniscus on the side of the sample bottle with a white grease pencil. The mark will be used later to determine the sample volume. Pour the entire sample into a 2 L separatory funnel.

10.2.2. The LCS and LCSD are defined as de-ionized water spiked with 100 µl of surrogate solution and 100 µl of spiking solution (see Section 6.12.) + 80 mg of sodium thiosulfate added to each in 1 Liter amber bottles same as sample collection bottles. Mark the meniscus on the side of the sample bottle with a white grease pencil. The mark will be used later to determine the sample volume. Pour the entire sample into a 2 L separatory funnel.

10.2.3. The MS and MSD are the designated batch QC sample spiked with 100 µl surrogate solution and 100 µl of spiking solution added to each. Mark the meniscus on the side of the sample bottle with a white grease pencil. The mark will be used later to determine the sample volume. Pour the entire sample into a 2 L separatory funnel.

10.2.4. During review of the Backlog Report, the analyst reviews the residual chlorine reported by the sample collector. See section 7.

10.3. Remove sample bottles, standards, and reagents from cold storage and allow equilibration to room temperature prior to sample preparation and/or analysis.

10.3.1. Mark the meniscus on the side of the remaining sample bottles with a white grease pencil. The mark will be used later to determine the sample volume. Add 100 µL of surrogate solution to each sample. Pour the entire sample into a 2 L separatory funnel.

10.3.2. Add 5.0 ml of pH 7 Di-potassium phosphate buffer to each sample and check pH with indicator test strips. If the sample pH is not neutral after the addition of the Di-potassium phosphate buffer, add 1 - 5 drops of H_2SO_4 (acid) or 0.1 - 0.5 ml of 10N NaOH (base) if necessary, until a neutral pH is achieved. Check pH with indicator strips after each adjustment.

10.3.3. Add 100 g of salt to each sample, seal, and shake to dissolve salt.

10.3.4. Rinse each sample bottle with 60 ml of methylene chloride. Finally, add this methylene chloride to the corresponding separatory funnel.

10.3.5. Shake the sample on a shaker for two minutes and allow for venting.

10.3.6. Allow the Methylene chloride to settle. Collect sample extract in a 250 ml Erlenmeyer flask or larger.

10.3.7. Add 60 ml of methylene chloride to each sample, shake for two minutes and collect the extract two more times. It is unnecessary to rinse the 1000 ml amber sample bottle after the initial rinse.

10.3.8. To concentrate, pour the extracts through a Sodium sulfate/glass wool drying column pre-rinsed with Methylene chloride and collect the extract in a RapidVap concentration tube or equivalent of at least 300 ml volume capacity.

10.4. Concentrate the extracts with nitrogen in a RapidVap to approximately 10 – 20 ml at 38°C with nitrogen pressure at 4psi and shaking rate of ~30 RPMs.

10.4.1. Transfer the extract from the RapidVap concentrator tube to a TurboVap tube or equivalent. Rinse the RapidVap tube with approximately 5 ml of MTBE and transfer to the TurboVap tube.

10.4.2. Gently swirl sample in the TurboVap tube to mix the solvents.

10.4.3. Concentrate the extracts with nitrogen to approximately 5 ml at 38°C with nitrogen pressure at 3-4psi.

10.4.4. Solvent exchange from Methylene chloride to MTBE by adding 5 ml of MTBE and repeat sections 10.4.2. and 10.4.3. Do not allow sample extract volume to reach less than 4 ml as some early eluting compounds may be lost. Manually check extract volume often and swirl extract each time.

10.4.5. Repeat section 10.4.4. two more times.

10.4.6. After the third solvent exchange, allow extract to concentrate to approximately 4 ml then transfer to culture tube of at least 5 ml capacity.

10.4.7. Rinse the TurboVap tube with 1 ml of MTBE and transfer to the culture tube, bringing the final volume of the extract to 5 ml using a pre-measured 5 ml MTBE culture tube model for comparison.

10.5. With a disposable pipette, transfer an aliquot of the extract to a 2 ml autosampler vial with a 300 µl insert, cover with screw cap, and analyze on a gas chromatograph with nitrogen-phosphorus detector.

10.6. Upon finishing the extraction of the samples. Determine the volume by filling up the sample bottles with water to the mark. Transfer the water into a 1000 mL class A graduated cylinder. Record the volume to the nearest 5 mL. (Note: It may be necessary to use smaller graduated cylinders if the volume happens to be over 1000 mL).

10.7. <u>Dilutions</u>

10.7.1. Upon analysis of the extract, if a target compound response is greater than that of the highest standard of the calibration curve, the sample must be diluted with the final extraction solvent (MTBE) so that, upon analyzing the dilution (in a valid analysis sequence), the target response is between the lowest concentration standard (or the reporting limit, whichever is higher) and the highest concentration standard.

10.8. PT Study:

10.8.1. Once in a 12-month period a PT study must be performed. An accredited testing facility will send the Laboratory an ampule for the compounds of interest listed in this SOP. The testing facility will send direction on how perform the dilutions necessary for the Analyst to spike into a sample. (Note: Please include a copy of instructions from the facility in the batch folder.)

11. Calculations

11.1. Response Factor, RF, for a peak

$$\mathrm{RF} = \frac{\mathrm{Area}_{\mathrm{Analyte}}}{\mathrm{Concentration}_{\mathrm{Analyte}}}$$

11.1.1. Where:
RF = Response Factor
$\mathrm{Area}_{\mathrm{Analyte}}$ = Area of the peak of the analyte of interest
$\mathrm{Concentration}_{\mathrm{Analyte}}$ = Concentration of the analyte of interest in µg/ml

11.2. Average Response Factor, $\overline{\mathrm{RF}}$

$$\overline{\mathrm{RF}} = \sum \frac{RF_i}{n}$$

11.2.1. Where:
$\overline{\mathrm{RF}}$ = Mean response factor
RF_i = Response factor of compound at each level i
n = Number of calibration standards

11.3. Sample Standard Deviation (n – 1) (σ_{n-1}) of response factors

$$\sigma_{n-1} = \sqrt{\sum_{i=1}^{n} \frac{(RF_i - \overline{RF})^2}{n-1}}$$

11.3.1. Where:
σ_{n-1} = Sample Standard Deviation
$\overline{\mathrm{RF}}$ = Mean response factor
RF_i = Response factor of compound at each level i

n = Number of calibration standards

11.4. First Order Linear Regression Response Equation

$$Y = ax + b$$

11.4.1. This rearranges to:
$x = Y - b/a$

11.4.2. Where:
Y = Instrument response
a = Slope of the line
b = Intercept
x = Concentration in the extract or standard

11.5. Second Order Quadratic Fit Equation

11.5.1. $Y = ax^2 + bx + c$

11.5.2. Where:
Y = Instrument response
a = Slope of the line
b = Intercept
c = constant
x = Concentration in the extract or standard

11.5.3. Subtract Y from c to get modified equation $0 = ax^2 + bx + c$

11.5.4. Solve for x using the quadratic formula:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

11.5.5 A positive and negative value will be generated. Use positive value.

11.6. Average Retention Time, $\overline{RT}$

$$\overline{RT} = \sum \frac{RT}{n}$$

11.6.1. Where:

$\overline{RT}$ = Mean retention time for the target compound
RT = Retention time for the target compound
n = Number of values

11.7. Percent Drift, %Drift

$$\%Drift = \frac{(\text{Concentration}_{\text{Calculated}} - \text{Concentration}_{\text{Expected}})}{\text{Concentration}_{\text{Expected}}} * 100$$

11.7.1. Where:

$\text{Concentration}_{\text{Calculated}}$ = Concentration calculated from result
$\text{Concentration}_{\text{Expected}}$ = Theoretical concentration of the standard

11.8. Extract Concentration Calculation (µg/ml)

$$\mu g/_{ml} = \frac{(A_s)}{(\overline{RF})}$$

11.8.1. Where:

A_s = Peak area of analyte
$\overline{RF}$ = Average Response Factor

11.9. Sample Concentration Calculation (µg/L)

$$\mu g/_{L} = \frac{C_s * 1000\frac{ml}{L} * V_t}{V_s}$$

11.9.1. Where:

C_s = Extract concentration in µg/ml
V_t = Extract volume in ml
V_s = Original sample volume in ml

11.9.2. Assuming an original sample volume of 1 L and an extract volume of 5 ml, equation 11.9. reduces to:

$$\mu g/_{L} = C_s * 5.0$$

11.9.2.1. Where:
C_s = Extract concentration in µg/ml

11.10. Sample Concentration Adjustment for Varying Initial Volume and Dilutions

$$\mu g/L_{Corrected} = \mu g/L_{Uncorrected} * \frac{(1000\ \text{ml})(\text{DF})}{V_s}$$

11.10.1. Where:
DF = Dilution Factor
V_s = Original sample volume in ml

11.11. Quality Control Calculations

$$\text{LCS/LCSD/ICV \% Recovery} = \frac{R_{spike}}{\text{Expected Result}} X\,100$$

$$\text{\% RPD(precision)} = \frac{\left|R_{sample} - R_{duplicate}\right|}{\left(\frac{R_{sample} + R_{duplicate}}{2}\right)} X\,100$$

11.11.1. Where:
R_{spike} =% recovery of spiked sample
R_{sample} =% recovery of sample
$R_{duplicate}$ =% recovery of duplicate sample

11.12. LPC Calculations

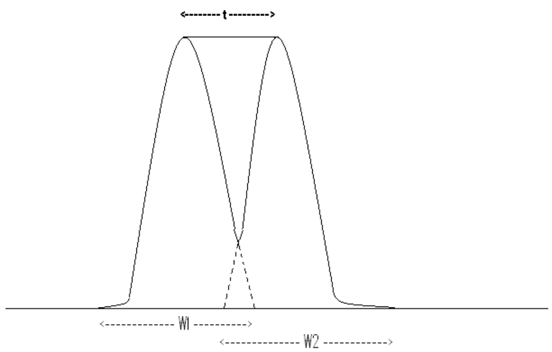
11.12.1. Sensitivity:

11.12.1.1. Instrument sensitivity is determined by comparing the LPC peak height of the weakest responding analyte, Vernolate, in the LPC to signal noise. The height of this peak must be 3 times greater than the largest noise peak.

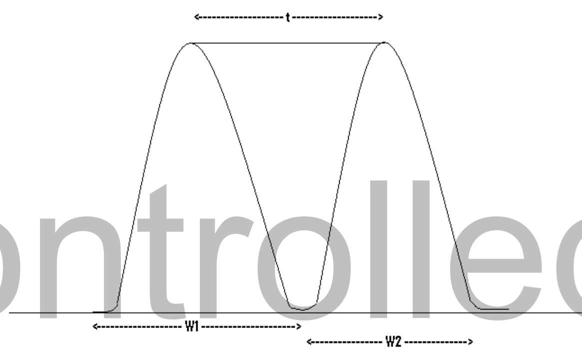
$$\frac{S}{N} = \frac{\mu V_{Analyte}}{\mu V_{Noise}}$$

11.12.2. Resolution (Column Performance):

11.12.2.1. Column performance is determined by calculating the resolution factor between the two closest eluting peaks, Prometon and Atrazine, in the LPC.



Column Performance - Peak Resolution for Overlapping Peaks



Column Performance - Peak Resolution for Resolved Peaks

11.12.2.2. Resolution between two peaks is determined by dividing the difference in the peak apex retention times by the average of the widths of the two peaks at the baseline. If the peaks overlap as in the second example above, estimate the peak widths as shown.

$$R = \frac{t}{\left(\frac{W1 + W2}{2}\right)}$$

11.12.2.2.1. Where:

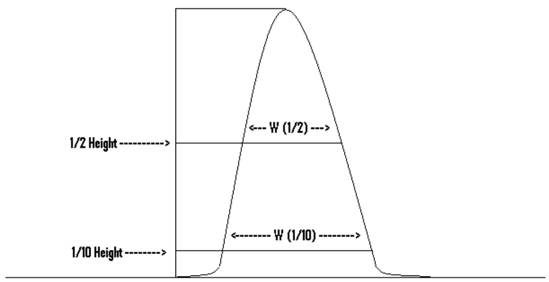
R=Resolution between two peaks

t = the difference in elution times between the two peaks

W1, W2 = the peak widths at baseline of the two peaks

11.12.3. Peak Gaussian Factor (PGF):

11.12.3.1. The PGF is determined using the worst shaped peak, Bromacil, in the LPC chromatogram. The PGF is equal to 1.83 times the ratio of that peak's half-height width and the tenth height peak width.



Peak Gaussian Factor - Measuring width at half peak height - W (1/2) and one tenth height - W (1/10)

$$PFG = \frac{1.83 * W_{1/2}}{W_{1/10}}$$

11.12.3.1.1. Where:

PGF = Peak Gaussian Factor
$W_{½}$ = the peak width at ½ height from base in seconds
$W_{1/10}$ = the peak width at $^{1}/_{10}$ height from base in seconds

11.13. Sample chromatograms generated from the processing software have calculation formulas already incorporated into the report format (see calculations 11.8. and 11.9.). Manual adjustments are required for diluted samples, or samples of other than 1000 ml only (see Section 11.10.). The RPD calculations are not incorporated into the report formats and must be calculated manually or using an Excel spreadsheet. If Excel spreadsheets are used, RPD results may be manually written on LCSD and MSD reports. LPCs must be measured with a ruler or other straight edge to mark time values on the chromatograms and calculated manually.

11.13.1. Alternatively, Total Chrom or equivalent chromatography software or the values on the Total Chrom LPC System Suitability Report may be used to calculate LPC results.

11.14. The LPC stock standards are typically certified standards at 1000 µg/ml concentration. Should an alternate concentration be required, or neat standard used due to availability issues, the calculations will be modified to meet EPA Method 507 LPC concentration requirements.

Table 11.14.1. 1 – 507 LPC Stock Standards in MtBE

Compound	Initial Concentration (µg/L)	Aliquot (ml)	Final Concentration (µg/ml)
Vernolate	1000	0.1	10
Bromacil	1000	1.0	100
Prometon	1000	0.1	10
Atrazine	1000	0.1	10

Total Volume of Standard Aliquots	0.1 or 1.0 ml (see above)
Addition of MtBE to Standard Aliquots	9.9 or 9.0 ml (see above)
Final Volume of LPC Stock Standard in MtBE	10 ml

Table 11.13.1. 2 – 507 LPC Standard in MtBE

Compound	Initial Concentration (µg/ml)	Aliquot	Final Concentration (µg/ml)
Vernolate	10	50 µl	0.05
Bromacil	100	500 µl	5.0
Prometon	10	300 µl	0.30
Atrazine	10	150 µl	0.15

Final Volume of LPC Standard in MtBE	10 ml

12. Waste Management

12.1. See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating procedures, SOP 6-015, online revision.

13. References

13.1. EPA/600/4-88-039 - EPA Method 507, Revision 2.1, 1995

13.2. GA EPD Laboratory SOP's- Initial Demonstration of Capability SOP 6-001, online revision and/or Continuing Demonstration of Capability SOP 6-002, online revision.

13.3. GA EPD Laboratory SOP- EPD Laboratory Procedures for Control Charting and Control and Control Limits SOP, SOP 6-025, online revision.

13.4. GA EPD Laboratory SOP- EPD Laboratory Waste Management SOP, SOP 6-015, online revision.

13.5. Manual for the Certification of Laboratories Analyzing Drinking Water, EPA/815-R-05-004, January 2005

13.6. GA EPD Laboratory SOP- Determination of Method Detection Limit, Method Detection Limit SOP 6-007, online revision.

13.7. GA EPD Laboratory Quality Assurance Plan, online revision.

13.8. GA EPD Laboratory Safety/Chemical Hygiene Plan & Fire Safety Plan, online revision.

14. Reporting Limits (RLs), Precision and Accuracy Criteria, and Quality Control Approach

14.1. Refer to Appendix A, Table A.1 for precision and accuracy criteria.

Table 14.1. 1 RLs for EPA Method 507

Parameter/Method	Analyte	Matrix (Water)	
		RL	Unit
EPA Method 507	Alachlor	0.44	µg/L
	Atrazine	0.22	µg/L
	Simazine	0.15	µg/L

Table 14.1. 2 Summary of Calibration and QC Procedures for EPA Method 507

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
EPA Method 507	Nitrogen-Phosphorous pesticides	4-point initial calibration for all analytes	Initial calibration prior to sample analysis	Linear-least squares regression $r \geq 0.990$ or $r^2 \geq 0.980$	Correct problem then repeat initial calibration	
		Second source calibration verification (ICV)	Once initial calibration or a minimum of once per quarter (see "Quarterly ICV" below	All analytes within ±20% of expected value	Correct problem then repeat initial calibration	
		Retention Time window calculated for each analyte	Once per year or after major maintenance that would affect RTs	± 3 times standard deviation for each analyte retention time for standard analytical batch sequence	Correct problem then re-analyze all samples analyzed since the last retention time check	
		Retention time window update	Updated with each calibration.	First ICV of each sequence and afterwards, the first CCC of each 24 hour period.		

Table 14.1. 2 Summary of Calibration and QC Procedures for EPA Method 507						
Method	**Applicable Parameter**	**QC Check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA Method 507	Nitrogen-Phosphorous pesticides	Initial calibration verification (CCC)	A CCC is ran at the beginning of each sequence prior to the analysis of samples. After the initial CCC an alternating CCC must be run every 8 hours and also at the end of the sequence.	All analytes within ± 20% of all expected value	Rerun once. If still out of range high, high bias with no detects, generate a corrective action and use data. If low bias or with detects, rerun CCC and affected samples. If rerun passes, use data. If reruns do not pass, correct problem, repeat initial calibration verification and reanalyze all samples since last successful calibration verification	
		Laboratory Performance Check	Prior to the analysis of each sample sequence	Signal to baseline noise ratio > 3, Resolution > 0.7 and peak Gaussian factor > 0.8 and < 1.2	Correct problem and re-analyze	
		IDC-Demonstrate ability to generate acceptable accuracy and precision using four replicate analyzes of a QC check sample, a Blind and a Blank. Analyst must also produce a passing MDL study with 7 MDL spikes and 7 MDL blanks.	Once per analyst	QC acceptance criteria Table A.1 Appendix A. **See section 9.9 for MDL requirements**	Locate and fix problem then rerun or re-extract demonstration for those analytes that did not meet criteria	
		CDC – Continuing Demonstration of Capability	Required every Six Months after IDC for each analyst	See Appendix A, Table A.1	Locate and fix problem then rerun or re-extract demonstration for those analytes that did not meet criteria	

Table 14.1. 2 Summary of Calibration and QC Procedures for EPA Method 507

Method	Applicable Parameter	QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria
EPA Method 507	Nitrogen-Phosphorous pesticides	Surrogate spike	Every sample, spiked sample, standard and method blank	QC acceptance criteria Table A.1 Appendix A	Analyze second extract aliquot, if this does not pass, correct problem then re-extract and re-analyze the sample	
		Method Blank Solvent Blank	One per analytical batch	No analytes detected >RL	Analyze second extract aliquot, if this does not pass, correct problem then re-analyze or re-extract the blank and all samples in the affected batch	
		LCS/LCSD for all analytes	One per analytical batch of 20 or less samples	QC acceptance criteria Table A.1 Appendix A	If an LCS/LCSD fail, it may be reran at least 24 hours from the original run or up to 12 hours from the end of the sequence. Then if the rerun of the LCS/LCSD result with a failure then all samples associated with the batch must be re-extracted.	Flag QC sample report if LCSD exceeds upper acceptable control limits with passing RPD when high bias with no detects
		MS/MSD	MS/MSD once per batch of up to 20 samples (minimum of 5% of all samples analyzed)	QC acceptance criteria Table A.1 Appendix A	Flag QC sample report	
		Second-column confirmation	100% for all positive results	If used for quantitation, same as for initial or primary column analysis	Same as for initial or primary column analysis	
		MDL study	Once per year or after major maintenance of the instrument	All Spiked MDLs must have a value greater than 0. Minimum Detection Limits established shall be < the RLs in Table 14.1	Re-do MDL Study	None

Table 14.1. 2 Summary of Calibration and QC Procedures for EPA Method 507						
Method	**Applicable Parameter**	**QC Check**	**Minimum Frequency**	**Acceptance Criteria**	**Corrective Action**	**Flagging Criteria**
EPA Method 507	Nitrogen-Phosphorous pesticides	MDL analysis	Once per batch or as needed to acquire data points per SOP 6-007, online revision	All Spiked MDLs must have a value greater than 0. All other QC in the MDL blank and MDL sample (i.e. Surrogate Spike or Internal Standard, etc. if included) must meet established criteria	Correct problem and re-run the MDL sample or MDL blank once and initiate a corrective action. If the re-run fails a second time, do not use MDL data. Update corrective action, and use associated sample data	None
		Results reported between MDL and RL	None	None	None	
		Quarterly ICV	Once per Quarter	All analytes within ± 20% of expected value	Correct problem then repeat calibration	
		Performance Test (PT sample)	Once per year			

15. Associated Labworks Test Codes

15.1. Parent Test Code

15.1.1. $507A – Analysis results

15.2. Extraction Test Code

15.2.1. 507E – 1 L amber glass bottle – Liquid/ Liquid extraction

15.3. QC Test Codes

15.3.1. $B_507A – Extraction Blank Results

15.3.2. $LA507A – LCS/LCSD Spike Amount

15.3.3. $LS507A – LCS Results

15.3.4. $LD507A – LCSD Results

15.3.5. $LR507A – LCS Percent Recovery

15.3.6. $L2507A – LCSD Percent Recovery

15.3.7. $LP507A – LCS/LCSD Precision

15.3.8. $A_507A – MS/MSD Spike Amount

15.3.9. $S_507A – MS Results

15.3.10. $D_507A – MSD Results

15.3.11. $R_507A – MS Percent Recovery

15.3.12. $RD507A – MS Percent Recovery

15.3.13. $P_507A – MS/MSD Precision

15.3.14. $MA507A – MDL Spike Amount

15.3.15. $ML507A – MDL Results

15.3.16. INSTR-507A – Instrument associated with batch

Appendix A, Table A.1 – Quality Assurance Criteria for Method EPA 507

QC Type	Analyte	Accuracy (%R) LCL		Accuracy (%R) UCL	Precision (%RPD)
LCS/LCSD*					
	Alachlor	65	-	110	25
	Atrazine	64	-	120	25
	Simazine	70	-	130	26
Surrogate**					
	NMX	70	-	130	NA
	NMX (as ug/L)	7.0	-	13	NA
MS/MSD**					
	Alachlor	55	-	115	30
	Atrazine	60	-	124	30
	Simazine	65	-	135	30

*LCS/LCSD recovery and precision limits based on control charts of data collected from 12/31/2018 to 01/01/2021 with a range limit of R ± 30%.

**Method 507 specifies 70 – 130% recovery limits for surrogates and R ± 35% for matrix spike analytes. The EPD Lab sets a static range of 0 – 30% for matrix spike precision.

Table 9.6.2 of the SOP provides information on how these default control limits are established.

Updates:

Appendix A added. Updated for online revision.